

The University of Chicago

THE INFLUENCE OF ROENTGEN-RAY
TREATMENTS OF THE HYPOPHYSIS
ON REPRODUCTIVE SYSTEMS OF THE
GROUND SQUIRREL AND RAT

A PART OF A DISSERTATION SUBMITTED TO
THE FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOOLOGY

1941

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THE INFLUENCE OF ROENTGEN-RAY TREATMENTS OF THE HYPOPHYSIS ON REPRODUCTIVE SYSTEMS OF THE GROUND SQUIRREL AND RAT

ROLLIN HENRY DENNISTON II

The University of Chicago, Department of Zoology, Illinois

TWO FIGURES

I. INTRODUCTION

The answer to the problem of the exogenous control of the pituitary gland is one of the most elusive and one of the most potentially valuable in the unexplored reaches of physiology. Following the work of Moore and Price ('32), we have come to realize that a reciprocity exists between the pituitary and other endocrine glands, particularly the gonads. Here, then, is a measure of endogenous control of the pituitary. However, the attempt to stimulate the pituitary through the peripheral members of the system is most unsatisfactory, either clinically or experimentally.

Application of x-ray to the pituitary has been reported in cases of ovarian dysfunctions associated with the menopause in the hope of "waking up" the hypo-active gland. Various early laboratory workers tried the effect of x-ray and radium treatments of the pituitary gland. Owing to lack of a standard technique and dosage, many of their findings are inconsistent. Pertinent reports will be discussed below.

The pituitaries of certain annual-breeding birds and mammals can be stimulated by changes in light (Rowan, '25, '26, '28, '29, '38; Bissonnette, '30 a, b, '31 a, b, '32, '33, '35 a, b, '36; Bissonnette and Chapnick, '30; and Benoit, '34, '35 a, b, c, '36 a, b, '37). However, in the ground squirrel, *Citellus tridecemlineatus*, studied by Moore, Wells, and co-workers

('34), neither light, temperature, diet, nor endocrine treatment stimulated the pituitary when the animal was in the sexually inactive phase (July to November).

On the other hand, the gonads and sex accessories of the quiescent-phase ground squirrel will respond to the gonadotropic principle and the appropriate sex hormone, respectively (Wells, '35). Thus, if it were possible to induce such a pituitary to renew its output of gonadotropic hormone, the effects would show on the reactive sex system. We proposed to investigate whether a light dose of x-ray might not furnish such a stimulus, hoping in this way to test the validity of the older theory of a "stimulating dose of x-ray."

The work with light doses of x-ray led to the problem of whether an animal whose pituitary had been subjected to relatively large doses of x-ray during the inactive season would be able to come into breeding condition the following spring; whether all types of anterior-lobe cells would be affected equally; or if a differential effect could be obtained with consequent graded effects on the end-point phenomena of the endocrine system.

To check the results in the annual-breeding rodent, the albino rat was treated similarly; and as a test of the biological effectiveness of the x-ray machine, as used, the testes of both species were rayed.

The problem under investigation was suggested to me by Prof. Carl R. Moore, and I am indebted to him for guidance and counsel during the course of the work. I am also under obligation to Dr. Sylvia Bensley for advice on the histological problems involved in this study.

This investigation has been aided by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

II. MATERIALS AND METHODS

The ground squirrels, *Citellus tridecemlineatus*, were captured in the field and either treated immediately, or confined

in laboratory cages until the appropriate time for treatment. Laboratory-confined animals undergo cyclic changes similar to those in nature, but may reach a given stage of sexual development approximately 2 weeks earlier than the outdoor animals (Wells, '35). The latter emerge from hibernation in a breeding condition about mid-April. By late June or July the reproductive system presents an almost castrate picture, remaining in this condition until November. In September or October, the animals go into hibernation. In late November, while the animals are still in hibernation, the gonads show mitoses in the germ line, with gametocytes making their appearance. The accessory organs also begin to grow, the whole process culminating in April.

The x-ray was administered from a large therapy tube in Billings Memorial Hospital. Focal skin spot distance (FSD) was 60 cm. The machine ran at 20 kilovolts, 4 milliamperes. Filters of $\frac{1}{2}$ mm. of copper and 1 mm. of aluminum were used. An ionization tube recorded 7 roentgen units (7 r.) per minute from this set-up. In later sections of the experiment the machine was adjusted to give 21 r. per minute. Doses over 300 r. were given in alternate 15 minute runs and rests. A maximum of 2000 r. was given on any one day.

The animals were anesthetized with Nembutal and fastened to the under side of a small table. The top of this table was lead. A small port allowed the rays to reach only the pituitary region. In later radiations of the testes, the animals were secured in lead and copper tubes, so that only the scrotum was exposed.

In judging the endocrine condition of an animal, all the following criteria were utilized: weight and histology of the pituitary, thyroid, adrenals, testes, seminal vesicles, prostate, Cowper's and bulbo-urethral glands, ovaries, uterus, and fallopian tubes. Vaginal and epididymal smears were also made. Only the pertinent material is included in the tables.

Since nothing was known as to the effectiveness of different doses of x-ray on the pituitary of the ground squirrel at various points in the annual cycle, it was necessary to provide

a varying set of treatments to investigate the many possibilities.

III. EFFECTS OF X-RADIATION OF GROUND SQUIRREL PITUITARIES

A. Immature animals — weak doses

In order to investigate any possible x-ray stimulation of a pituitary that might be revealed by increased gonadotropic secretion, a group of immature male and female ground squirrels was rayed on the pituitary in November. Control animals should not show normal seasonal regeneration of the sex complex for about 60 days. Autopsies were performed from 3 weeks to 3 months after raying, a range which should be adequate to indicate any possible stimulative effect on the gonadotropic function of the anterior pituitary.

Thirty animals were used. Dosages ranged from 7 r. to 140 r. in steps of 7 r. The dose was administered in two ways: all at the same time, or 7 r. on successive days.

At autopsy the treated animals were in no way different from the controls. The males showed spermatogenesis, accessory enlargement, scrotal pigmentation, and descent of the testes within the normal time range of the laboratory controls. Weight and histology of testes, seminal vesicles, prostate, adrenals, pituitary, ovaries, uterus, and fallopian tubes demonstrated a similar lack of difference. The females showed vulvar swelling and cornified vaginal smear to the same extent as the controls. In brief, the animals came into breeding condition at the same time as the controls.

From this group of thirty animals, a few points stand out clearly. First, the pituitary gland of immature male and female ground squirrels, exposed to doses of 7 r. to 140 r., is most definitely not stimulated to secrete gonadotropic substances in sufficient amounts to become detectable by response in the reproductive system. Second, exposures of the pituitary to these relatively weak doses of x-ray cause no delay in the development of the cyclic maturity phenomena so characteristic of the reproductive system of this animal.

B. Immature animals — heavy doses

Since the weaker amounts of x-ray applied to the pituitary gland failed to show either a stimulating or a depressing effect on the reproductive system, three additional groups of immature animals were subjected to heavier irradiation. Dosages ranged from 500 to 4000 r., the highest employed on an immature animal. The time of application and autopsy was varied to cover different periods of the annual cycle. The x-ray machine was adjusted to give 21 r. per minute.

1. The first group is composed of four treated males and four treated females, with a normal control of each sex. They were rayed in October at a time of complete reproductive system inactivity. Their autopsy was arranged to coincide with a period when renewed sexual system developments would be first beginning.

TABLE 1

Immature male and female ground squirrels x-rayed on the pituitary in October, autopsied 52 days later.

Maximum weight loss		(Per cent of initial body weight)		
X-ray dose	550 r.	1000 r.	1500 r.	2000 r.
Male	—10%	—8%	—30%	—51%
Female	0 (+5%)	—11%	—2%	—34%

In males, testicular weight deviations were not pronounced and their histological condition was in no appreciable manner different from that of the controls. The evidence from weights of accessory reproductive organs demonstrates likewise that hormone secretion by these gonads also was modified to no significant extent. Ovarian tissue was normal histologically, and female genital tract dimensions and weights for the rayed animals were within the normal range for this period of the cycle.

Indeed, the rayed animals showed but one important difference from the controls (table 1). It was noted that a sharp loss in body weight occurred at the higher doses. The

weight and general physiological condition returned to normal within a month.

To investigate more closely the conditions existing at the time of greatest weight reduction, a second group of immature animals, consisting of seven treated and two control males, was rayed with 500 r. to 4000 r., the largest amount used on any immature animal.

TABLE 2

Immature male ground squirrels x-rayed on the pituitary November 2nd, autopsied November 13th.

Maximum body weight loss (per cent of initial); and testis weight of treated animals in per cent of control testis weight

X-ray dose	0 ¹	500 r.	1000 r.	1500 r.	2000 r.	4000 r.
Body weight	—	—10%	—8%	—23%	—41%	—48%
Testis weight	—23.2%	+7%	+1%	—23%	—27%	—36%

¹ Control autopsied on date of treatment.

At autopsy, 11 days after treatment, animals which had received over 2000 r. weighed 40% less than they did at the time of raying. Testis weight in all animals receiving more than 1000 r. shows a sharp reduction compared with the control. That in the case of the testis it is an interference with the seasonal growth that is just beginning at this time, rather than a reduction from the weight at the time of raying, is demonstrated by the fact that the testes of these animals weigh the same as those of the control, autopsied at the time of treatment. Nothing can be told from this experiment about the effect of the treatment on sex hormone output. The low weight of the accessory organs at the beginning of the experiment indicates that sex hormone must be at a minimum level at this time. The average weight of prostates of both treated and control animals was 8.29 mg., with only a small deviation. No destruction of the germ line or the interstitial cells was apparent, though the rayed animals were almost completely lacking in secondary spermatocytes, which were seen in the controls. A longer time would be necessary to see whether the effects on the gonads would be reflected in the later-

developing accessories, and what degree of recovery may be expected.

3. The third group of immature animals consists of both males and females, rayed on the pituitary with 2000, 2500, and 3000 r. These animals were rayed at the beginning of reproductive recrudescence and autopsied in March at a time when normal laboratory control animals are in full breeding condition. It was planned to demonstrate whether or not the treatment given at the beginning of the annual development would slow down its final culmination in the spring. Both sexes show a slight loss in body weight, but the females recovered from it within this 80-day period, while the males did not. On the other hand, male gonads and accessory organs were somewhat heavier than those of the control, while in the females this order was reversed. These differences are probably not significant in the light of histological findings.

Control and experimental testes were alike in having normal detritus in the lumina of the seminiferous tubules, sperm heads, Sertoli cells, and motile sperm in the epididymis.

The control ovaries of the parallel female series contained several corpora lutea, while the experimental ovaries had only fluid-filled follicles and many small follicles at the periphery. Both conditions are found in normal animals at this time of year. X-radiation of immature ground squirrel pituitaries with amounts sufficient to lower the animal body weights, then, does not significantly delay the onset of a full breeding condition the following spring.

*C. Adult male ground squirrels rayed
at reproductive maximum*

Since x-ray to the pituitary in the amounts given cannot prevent or delay the onset of a maximum reproductive condition, we were interested in seeing what pituitary irradiation during this maximal condition would do to the sexual system. A group of four adult male ground squirrels was x-rayed with the results shown in table 3. The animals were autopsied 1 month after raying.

A significant difference is seen between the weights of the testes, seminal vesicles, and prostates of the experimental animals. The untreated animal showed only a 5% difference in weight between the testis removed at the beginning of the experiment and that removed at the end. Compare this with 87% difference shown between the testes of the animal which received 4800 r. The rayed animals had no sperm in the epididymal smear, while the control showed motile sperm. From the results of other experiments, it seems possible that these differences represent an interference with the hormone of a pituitary which had almost stopped secreting, but it is a

TABLE 3

*Adult male ground squirrels x-rayed on the pituitary April 30th
and autopsied May 30th.*

(One testis was removed prior to irradiation)

X-ray dose	0	1200 r.	3600 r.	4800 r.
Testis ¹	—5%	—73%	—85%	—87%
Testis ²	...	—59%	—82%	—74%
Seminal vesicle ²	...	—94%	—97%	—98%
Prostate ²	...	—86%	—93%	—93%

¹ Per cent reduction compared with animal's own testis removed before treatment.

² Per cent reduction compared with control.

very real interference, whatever its ultimate nature. These adult animals did not suffer the loss in body weight seen before in rayed immature animals.

In summary, x-ray to the pituitary in the relatively high dosage used neither prevents nor accelerates the normal development of the sex complex in accordance with the annual cycle of the experimental animal. This is true if the treatment is given in October, November, or December at the time when the whole sex system is developing rapidly. On the other hand, when gonads and accessory organs have reached their annual peak in March and April, x-ray to the pituitary in doses over 3000 r. does seem to accelerate the descent from this peak.

IV. EFFECTS OF X-RADIATION OF THE PITUITARIES OF ALBINO RATS

Since the animal used in previous experiments was of an annual-breeding type, it seemed advisable to check the results obtained, by raying the pituitaries of constant-breeding albino rats. The animals used in any one series were litter mates.

A. Young puberal rats

As with the ground squirrels, it was first necessary to explore the possible effects of varying doses of radiation. For this purpose a sexually active age of the animal was desirable. A litter of 90-day-old male rats was exposed to 1000 to 3000 r. All animals were autopsied 3 weeks after treatment. There were no significant differences between the weight and histology of testes, seminal vesicles, prostates, coagulating glands, and Cowper's glands of the treated animals and those of the untreated controls. All the treated animals had sperm in the epididymides. No body weight loss had taken place.

Evidently the range of dosage used is unable to affect either the body weight of these animals or the gonadotropic function of the anterior pituitary.

B. Adult rats

Since a higher range acting for a longer time appeared necessary, a litter of adult male rats was rayed with 3400 to 6000 r. A maximum of 2000 r. was given on any one day, and all treatments were given within 1 week. The animals were autopsied 80 days after the last treatment.

TABLE 4

Adult male litter-mate albino rats x-rayed on the pituitary, autopsied 80 days later.

X-ray dose	PER CENT REDUCTION COMPARED WITH CONTROL		
	Testes	Ventral prostate	Other accessories ¹
3400 r.	—5%	12%	—4%
4000 r.	0%	—7%	—61%
6000 r.	—48%	—13%	—54%

¹ Seminal vesicles, coagulating gland, and Cowper's gland.

The animals rayed with 6000 r. and 4000 r. are particularly interesting. The first had testes weighing about one-half those of the controls and the rest of the experimental animals. It was the only animal which lacked sperm in the epididymis at autopsy. Both had Cowper's glands, seminal vesicles, and coagulating gland significantly lower in weight than those of the other animals. However, the prostates showed no variation throughout the dosage range.

At 3400 r. approximately one-half the seminiferous tubules were empty. At the higher doses, destruction was more apparent, and at 6000 r. the interstitial material was somewhat pyknotic. That these changes are reflected in the pituitary cytology will be noted in a later section of this paper.

In the neighborhood of 5000 r. we have that dose which, when applied to the pituitary gland of the rat, will cause definite effects in the gonads. The effect on the germ line is obvious; that on the hormone-producing elements of the testes would seem to be less so, since the prostate is not affected.

C. Pre-puberal rats

1. Cytological examination of some x-rayed pituitaries, to be discussed later, showed effects on those elements which elaborate the growth-promoting hormone. To investigate the physiological effect of this alteration, a litter of 26-day-old rats was rayed in the pituitary region with 1000, 2000, and 3000 r. They were autopsied at the end of the third week.

TABLE 5
*Twenty-six-day-old litter-mate albino rats x-rayed on the pituitary,
autopsied 21 days later.*

X-RAY DOSE	GROWTH RATE (% of original per day)	TESTIS WEIGHT % of Control 2	ACCESSORY WEIGHT ¹ % of Control 2
Control 1, Beginning of Exp.		—84%	—88%
Control 2, End of Experiment	11.78%		
1000 r.	9.09%	—25%	—71%
2000 r.	6.31%	—52%	—83%
3000 r.	7.00%	—63%	—76%

¹ Seminal vesicles, coagulating glands, and ventral prostates.

Although the treated animals did show some body growth and reproductive development from the condition represented by the control taken at the beginning of the experiment, such growth was negligible compared with that of the control animal taken at the end of the experiment. In extreme cases, the gonads weighed a third as much and the accessory glands an eighth as much as those of the litter-mate control. No sperm were found in the epididymal smear in animals receiving more than 1000 r.

Of two females in the litter, one was autopsied on the day the other was rayed with 3000 r. In the 3 weeks that elapsed before the treated animal was autopsied, no increase in ovarian weight had taken place, and only a very slight increase in the weight of the whole genital tract.

The suggestions of a possible growth rate reduction inferred from the histological study of rayed pituitaries are borne out by this experiment. Furthermore, we see that only 2000 r. affect the reproductive system of these young animals, whereas 5000 r. had been necessary to affect the adult system.

2. It has been shown that x-radiation of the pituitary in 26-day-old rats has definite inhibitory effects on the growth rate. To find out whether still growing, but somewhat older animals were equally susceptible to the effects of x-ray, a litter of 40-day-old rats was subjected to 1000, 3000, and 6000 units and autopsied 40 days later. Loss in body weight by the treated animals, though marked, was not so extreme as in the group rayed at an earlier age. The average body weight of the experimental animals at the end of the experiment was 155.55 gm.; that of the control group, 187.30 gm. Motile sperm were seen in the smears of all males at autopsy, excepting only that animal which had been exposed to 6000 r. There was little difference in the weight of gonads and accessories of the treated and control animals, indicating a greater resistance of these structures, or of the pituitary itself, than found in the younger group. One female was rayed; the other was not. Since the litter was left together, these females were pregnant. The embryos from the treated animal were in the process of

resorption, while those in the control uterus were perfectly normal. Both females had apparently normal corpora lutea of pregnancy. Two animals in this litter, exposed to 8000 r., died in the night following the last dose.

Several points stand out from this experiment. As the animal grows older, the susceptibility to x-ray, as to other agents, decreases. The resistance of the gonadotropic hormone-producing cells has especially increased, as compared with the 26-day-old animals. For the first time, animals died as a result of x-radiation of the pituitary — at 8000 r.

V. EFFECT OF X-RAY ON PITUITARY CYTOLOGY

A. Ground squirrel pituitaries

Pituitaries from the series of animals 91–98 $\frac{3}{4}$ ¹ in table 1, which had been subjected to 550–2000 r., 51 days before autopsy, were sectioned at 3 micra and stained in Azan-Mallory. The normal controls for this series contained typical granular basophiles and acidophiles in the ratio of 1:2.

With increasing doses of x-ray the basophiles of the anterior lobe and the posterior lobe tissue are unaffected. However, the acidophiles and the blood sinuses of the whole gland do show some progressive changes. The cytoplasm of the alpha cells becomes muddy, the cell wall becomes ruffled or scalloped, while the characteristic orange granules disappear. The cytoplasm appears to shrink in volume, while the nucleolus increases in size until it almost fills the nucleus. There is also a progressive decrease in number of acidophiles. The blood sinuses become increasingly sclerotic to the point of partial occlusion of their lumina. At the same time, they become more engorged with red blood cells, so that in animal 94 (2016 r.), they are very prominent, whereas in the control they may be made out only with care. There is no sex difference to be noted in the reaction of these pituitaries to x-radiation.

¹ Figures 1 and 2 are photomicrographs of pituitaries 94 and 98 $\frac{3}{4}$, respectively.

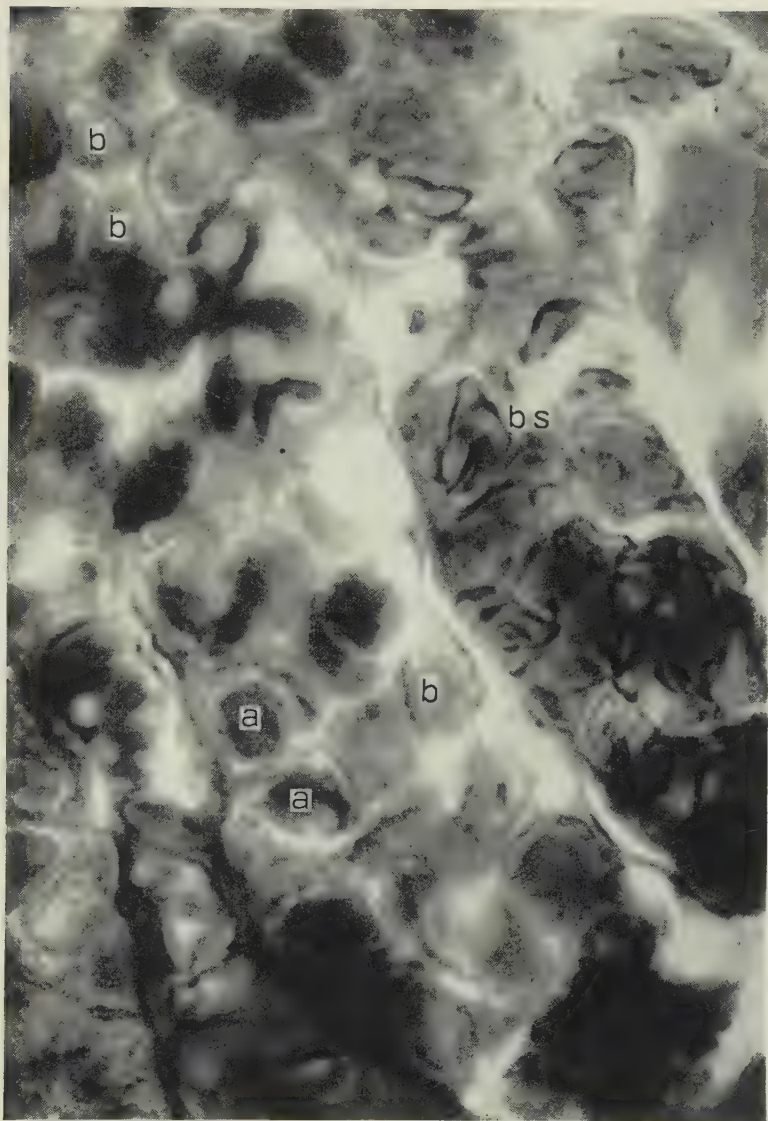


Fig. 1 Pituitary 94 ($\times 2600$). Exposed to 2016 r. on October 29th, autopsied December 21st.

a — acidophiles

b — basophiles

b.s. — blood sinus

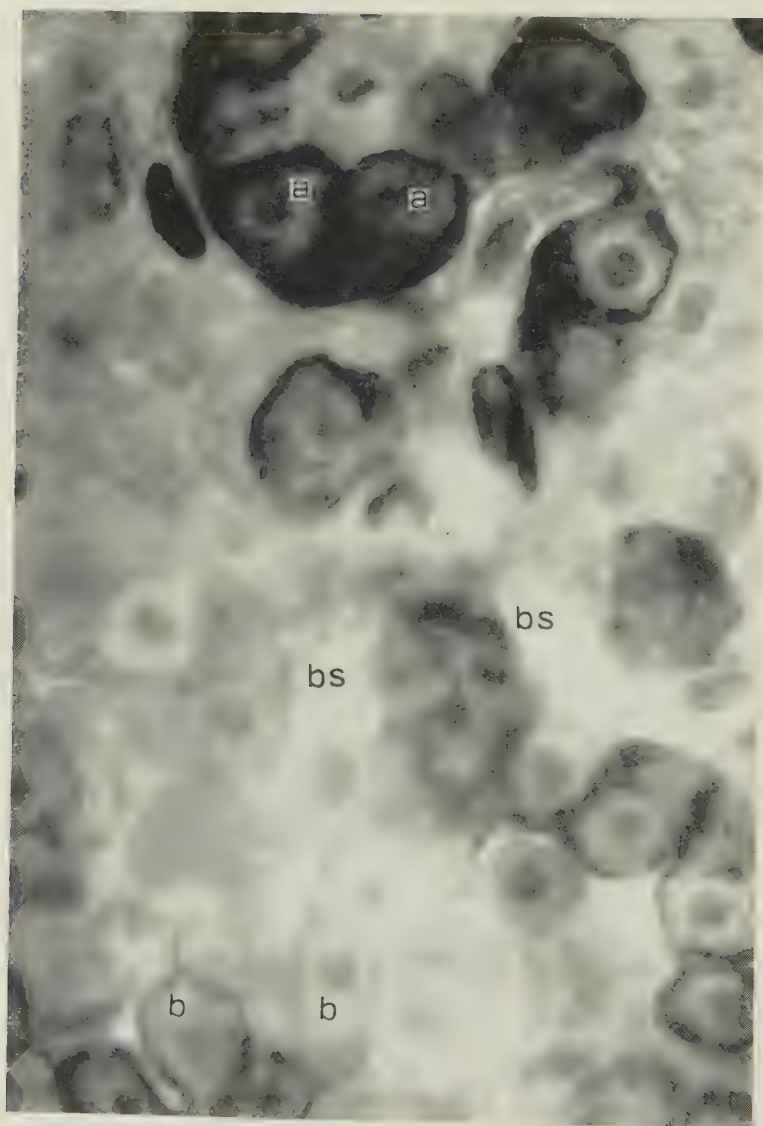


Fig. 2 Pituitary 981 ($\times 2600$). Untreated control autopsied December 29th.
a — acidophiles b — basophiles b.s. — blood sinus

These findings were borne out by studies of further such series of rayed pituitaries. The most constant features were the shrinkage of the acidophile cytoplasm, the increase in size of the nucleolus, and the sclerotization of the blood sinuses.

These radical effects on the acidophiles suggested that the growth-promoting hormone might be interfered with. That this was indeed the case was proved by the depressed growth rate of the litters of young rats rayed to the pituitary as a check on these cytological findings.

B. Rat pituitaries

As a check on the results on the annual-breeding rodent, pituitaries of the constant-breeding laboratory rat were irradiated and studied. Again, the shrinking of the acidophile cytoplasm, with loss of the typical granules, was noted. In animals receiving higher doses, notably the series of animals in table 4, the basophiles are affected. In the pituitary of an adult male rayed with 3400 units 80 days before autopsy, there are large, colloid-filled basophiles as well as possible transition phases of these to a typical "signet ring castration cell." This cell has the nucleus pushed to the periphery by the large central vacuole. With the Azan-Mallory stain the negative Golgi pattern is especially prominent. These end stage signet-ring cells are increasingly common as the dosage is raised; at 6000 units, approximately one-half the basophile cells are of this type. Although these cells are present from 3400 to 6000 r., motile sperm are found up to the 6000 unit level. This does not mean, of course, that gonadotropic hormone was unaffected till this point was reached, since a certain threshold level of depression is probably necessary to show the end-point in question. Why the basophiles are not similarly affected in the ground squirrel pituitaries is difficult to say, except that in those most carefully studied the radiation was carried on in a season when the basophiles and testes were physiologically quite inactive.

VI. EFFECTS OF X-RAY ON TESTES

One of the first biological effects of x-ray known was the effect on gonads. As a check on the biological effectiveness of the x-ray machine, as used, the testes of both the experimental species were rayed. How would the testes of the annual-breeding form react to x-ray administered at various times of year? How much difference in susceptibility would there be between the inactive gonads and the active ones of the same species on the one hand, and of a constant-breeding species on the other? We hoped to gain some insight into these problems by the experiments described below.

A. Ground squirrel testes

1. A group of adult male ground squirrels was unilaterally castrated early in April, x-rayed in the scrotal region with 100–2100 r., and autopsied 1 month after irradiation. Except for the animal which received the highest dose, there is no statistically significant difference between the means and coefficients of variation of any of the gland weights of the controls as compared with the rayed animals. There were motile sperm in the epididymides of all animals.

TABLE 6

Histology of three types of testes from active-phase ground squirrels, scrotum rayed with 100–2100 r.

TYPE OF TESTES	TUBULES	SPERM HEADS	PROPHASE SPERMATOGONIA
1. Before x-radiation	very large	many	many
2. Control at autopsy of 3	small no lumina	none	many
3. X-rayed testes	debris artificial lumina	none	few

Evidently x-ray is able to cause the expected destruction of these active testes, even though the weight lost so closely parallels the normal seasonal decline as to appear insignificant.

2. To see the effect of x-ray on quiescent phase testes, the following two experiments were planned. The animals were

rayed at a time when sperm were not present in the epididymis and the accessories were in a semi-castrate condition. In the first of the two groups, the dose was varied from 200 to 3200 r. and autopsy was performed 10 days after treatment. This amount of x-ray acting for this period of time on inactive testes is unable to depress further the weights of the gonads and of all the accessory organs except the seminal vesicles, which were sharply reduced by doses above 420 r. The average seminal vesicle weight for the treated animals was 76.3 mg., while the control glands averaged 120.1 mg. They have been shown by other workers to be extremely susceptible to hormone effects.

No histological difference is apparent between these experimental gonads rayed with as much as 3260 r. during the inactive phase and those of untreated controls. The seminiferous tubules of all contain spermatogonia, principally, with a few primary spermatocytes. These animals had all been autopsied 10 days after raying with varying doses. It was felt that perhaps this interval was too short to show any possible effects; so a group of similar ground squirrels was rayed with a uniform dose of 2520 r., and autopsied at 10-day intervals up to 40 days after raying.

The control testes and seminal vesicles are all slightly heavier than those of the rayed animals autopsied at the same time, except the animals autopsied at 40 days. However, weights of prostates, both experimental and control, show only the normal seasonal regression.

This series shows some interesting histological trends. An experimental animal killed on day 20 has gonads with a heavy connective tissue capsule. The seminiferous tubules are much flattened, and contain only spermatogonia in the germ line. Many of these have pyknotic nuclei. The control sacrificed at the same time was infected with nematodes. The capsule was not nearly so heavy, the nuclei in the germ line were not pyknotic, and there were sperm tails present. At 30 days the gonads of the experimental animal looked much like those of the rayed animal killed 10 days before. The control for this

animal had the capsule around the testes, but the seminiferous tubules were not flattened, as in the treated animal. By 40 days, the treated testes have a less dense capsule, and the tubules contain many primary spermatocytes in prophase, resembling in every way the control sacrificed on the same date. Evidently, gonads in the quiescent phase are not in a minimum condition. Even in this inactive state the pituitary secretes enough gonadotropic hormone to bring about recovery of the testes from x-ray destruction in 40 days.

B. Rat testes

To obtain data for comparison with those obtained in ground squirrel experiments of a similar nature, we x-rayed the testes of those rats described in table 7.

TABLE 7
Adult male rats x-rayed on the testes, autopsied 1 month later.
Per cent reduction compared with gland of second control

X-ray dose	First control	400 r.	800 r.	1200 r.	1600 r.
Testes	—37%	—51%	—67%	—70%	—72%
Seminal vesicles	—74%	—35%	—7%	+3%	—34%
Ventral prostate	—65%	—44%	—22%	—16%	—65%

The first control was sacrificed on the day the others were rayed; the second, the same day as the others. The rat receiving 400 r. pulled one testis under the lead shield while being x-rayed. This testis weighed 1307.9 mg., while the other testis on the same rat weighed 670.4 mg. If we take double the weight of the affected gonad (1340.8) as a corrected reading for this animal, we find a close correlation between amount of x-ray administered and loss of testis weight. Some accessory glands may have been within range of the x-ray and some not. If this were not so, their comparative weights would suggest that the dose of 1600 r., the highest given, arrested the growth of these structures at a relatively early stage.

Rat testes rayed with 400–1600 r. show a contrast with treated ground squirrel testes. There were no signs of re-

covery of the germ line when autopsied 30 days after raying, even with 400 r. It was interesting to observe that the other testis of this animal, which he pulled under the lead shield while being treated, was normal in every respect. All the irradiated testes showed destruction of the germ line back to the spermatogonia, and only one-hundredth of observed sperm in the epididymal smear were motile. The animal which was rayed with 1600 r. had no motile sperm, and only a few of any kind in the smear.

No "signet-ring castration cells" were noted in the pituitaries of any of the animals with irradiated testes.

Several points arising from the testis irradiation studies appear to be noteworthy. Active testes of both the ground squirrel and rat are susceptible to relatively low doses of x-ray. A reaction is shown to 400 r. in the case of the ground squirrel. The quiescent phase ground squirrel testes show no sign of reacting until about six times this dose, or 2400 r., is applied.

VII. DISCUSSION

The early literature in this field has been excellently summarized in the papers of Fehr ('36), Lacassagne and Nyka ('34 a), and Lacassagne ('35). A brief abstract of these summaries will introduce the history of the subject. The first report of x-radiation of the pituitary appears in 1920. Brunner, Geller, and Strauss (Fehr, '36; Lacassagne, '35) rayed the pituitary region of dogs, guinea pigs, and rabbits. Brunner and Strauss reported that the pituitary is only slightly sensitive to x-ray. Geller thought he saw some changes in the rayed pituitaries of young rabbits.

The following year, Geller (Fehr, '36; Lacassagne, '35) observed weight loss, growth retardation, and hypoplasia genitalis following hypophyseal x-radiation. He was not sure whether the effect was working through the pituitary or through some center in the brain.

In 1922 Hofbauer and Hirsh (Fehr, '36; Lacassagne, '35) first described "hypophyseal castration." It was in this year

that Poos, Rahm, and Ghilarducci (Fehr, '36; Lacassagne, '35) published on the subject.

Following 1927, many other European workers entered the field. Although they used nearly the same technique and experimental animals, namely, dogs and rabbits, they report widely different results. Podljaschuk, Poos, Del Buono, Rivoletta, Vischia, and Geller (Fehr, '36; Lacassagne, '35) find no alteration of the pituitary following irradiation. Ghilarducci obtained complete destruction of the pituitary, while Cannavo and Beniato (Lacassagne and Nyka, '34 a; Lacassagne, '35) achieved a fatal effect in most cases. Geller, Rahm, Ghilarducci, Podljaschuk, and Poos agree that x-ray to the young rabbit pituitary is followed by a reduction in growth rate. All but Poos and possibly Geller trace this to an effect from the pituitary. Poos attributed the growth rate reduction and hypoplasia genitalis to a general somatic effect, since he saw no histological changes in the hypophysis. It must be remembered that these earlier workers used machines which were inefficient by modern standards; moreover, they had no accepted dose standardization, so that there was no basis for comparison of the results of one worker with those of another.

Lacassagne and Nyka ('34 a) and Lacassagne ('35) reported destruction of the rabbit hypophysis by implants of radium "seeds." Associated with pituitary necrosis they observed loss of sex drive and gonad atrophy. No differential susceptibility of any type of pituitary cell was observed (Lacassagne and Nyka, '34 b). Pituitary tissue was the most resistant of any they exposed to radium. At no time did they observe mitosis. They obtained similar results with the cock, noting loss of psychological and physiological secondary sex characters associated with testicular atrophy. The germ line was completely gone in 1 month (Lacassagne and Nyka, '35). In 1935, Lacassagne found that female rabbits entered a spayed condition within a month after radium implantation.

Fehr ('36) noted an age differential effect of x-radiation of the pituitary in 1936. Inhibition of sex development, bone growth, and general body growth he laid to the intermediacy

of the pituitary. He administered doses up to 8270 r. to growing rabbits, in amounts of 600 r. The animals remained normal, physiologically and histologically. However, when 2860 r. were given at once, a slight cachexia developed, moderate weight loss followed, and the female refused to accept the treated male, who did not lose the sex drive. The interstitial cells of the ovary appeared juvenile and the uterus was small. The pituitary seemed normal histologically.

In 1937, Franck x-rayed the hypophysis and observed the effects on the thyroid and on metabolism. This author rayed guinea pigs with 25–1000 r. The animals were sacrificed 5 to 10 weeks after the raying. Above 300 r., the BMR showed a decline below normal. Thyroid secretory cells were flat, no Golgi could be seen, and colloid was present. There had been a decrease in acidophiles in the anterior pituitary, while the basophiles remained unchanged.

In 1937, Engelstad and Torgerson ('37) applied x-ray to rabbit adrenals. They observed a differential destruction of tissue. Degeneration and hyperemia were most marked in the fascicular and reticulate zones of the adrenal cortex.

The excellent paper of Lawrence, Nelson, and Wilson ('37) treats of hypophyseal radiations of juvenile female rats. They used two 1000-unit doses 2 days apart, and observed an inhibition of growth rate for 10 days, after which a normal rate was resumed. The effect on the endocrine system resembled that of a sub-total hypophysectomy. Associated with the repression of growth rate was a 70% reduction in acidophiles. Basophiles were reduced in size, a finding directly opposite to mine on rayed pituitaries studied after a longer interval. Thyroid, adrenal, and reproductive system reduction followed, with recovery starting within 27 days.

My findings, with the exception noted, are in complete agreement with those of the last three workers.

Medically, x-radiation of the pituitary and other endocrine glands has been used with varying degrees of success for a wide assortment of conditions. It is in the medical literature

that we find the suggestion that small amounts of x-ray may stimulate a hypo-active gland to renew its function.

Not one of the experimental laboratory workers cited so much as suggests the possibility of a "stimulating dose of x-ray." Certainly none of the experimental literature would support such a thesis. This paper has dealt, in part, with an attempt to stimulate one of the most inactive glands known, the pituitary of the ground squirrel, *Citellus*, in the sexually quiescent part of the annual cycle. No stimulation of this pituitary by x-ray was observed at any time, either within the gland itself, or in the effect of its hormones on the end-point glands and structures associated with it.

An attempt to inhibit pituitary function by x-ray was equally fruitless in the ground squirrel with one exception. The group of males rayed on the pituitary at the end of the active period showed an accelerated decline of the sex complex. This is probably an interference with the stored hormone in a pituitary which had already stopped secreting. Since irradiation of the pituitary at the beginning of its active period fails to affect the secretion of its gonadotropic hormone, it hardly seems possible that it would be able to do so toward the end of that active period. It may be that a hormone similar to luteotropin is necessary to maintain the active condition of the testis, and the x-ray interfered with the secretion of this hormone.

The fact that x-radiation of the adult rat hypophysis, in excess of 5000 r., and of the young rat hypophysis with 2000 r. and over, has definitely retarded sex development suggests that the lack of results in the ground squirrel is due to differential species susceptibility and to the fact that the highest dose given this animal was only 4000 r., while it takes 6000 r. to give effects on the sex system of the adult rats in this colony.

Contrary to many of the early workers, I find a very definite effect of x-ray on the pituitary itself, confirming and extending the work of Franck, and to some extent that of Lacassagne

and Nyka. Franck reports a decrease in acidophiles, but says that the cell form is preserved. The former is true with my ground squirrel material, and the latter partially so. The acidophile cytoplasm becomes degranulated and shrinks, giving the cell boundary a scalloped appearance. Perhaps this is the "hyalinization" to which Lacassagne refers. The "collagenous network" they find as a residue following the corroding effect of the β rays of radium probably corresponds to the walls of the blood spaces of the anterior lobe, which become increasingly sclerotic, until the lumen is practically occluded. While radium acts as a kind of cautery, giving complete destruction of the pituitary with results comparable to hypophysectomy, and never a differential effect, Franck ('37) and I, working with x-ray on the pituitary, and Engelstad and Torgerson ('37) working on the adrenals, are able to report such differential susceptibility. With x-ray treatment, then, the alpha cells are seriously interfered with, while the beta cells remain sound, in an animal whose gonadotropic hormone is apparently normal, but whose growth hormone may not be.

That the growth hormone output of the anterior pituitary may be seriously depressed by x-radiation was confirmed by the reduction to 50% of normal growth rate by radiation of 26-day-old rat pituitaries. The effect on growth rate took place at levels of dosage which did not affect sexual development, although this was easier to influence in these young animals than in the adults.

The destructive effect of x-ray on the developing germ line has long been known. It is of some interest, then, to observe that x-ray in doses under 3000 r., acting over a period of 10 days, is unable to depress further the already inactive germ line in ground squirrels. Starting at 20 days, destruction appears. Perhaps owing to the inactivity of the tissue involved, and the very heavy connective tissue capsule which covers the testes at this time, destruction is never so marked as in rat testes rayed with less than one-sixth the ground squirrel

dose. Their testes are able to start recovery from x-ray to the amount of 2500 r. within 40 days. This is in line with the findings of Wells ('35), who states that castrate ground squirrel accessory organs are in an appreciably lower state than merely seasonally inactive glands.

Evidently, x-ray affects tissue in very direct accordance with the thesis of Professor Child concerning the dependence of the susceptibility of a tissue on its comparative metabolic activity. Cancer tissue has one of the highest mitotic and metabolic rates known, and is extremely susceptible to x-ray. Next in order are the rapidly dividing cells of the germ line. This tissue is interfered with by direct doses over 500 r. The pituitary, which shows mitotic activity almost never, is generally unaffected by x-ray in amounts less than 2400 r. This would indicate an enormous threshold difference in metabolic activity between a tissue, secreting no matter how rapidly, and a tissue undergoing mitosis.

VIII. SUMMARY AND CONCLUSIONS

The animals used in this paper are the annual-breeding ground squirrel, *Citellus tridecemlineatus*, and the albino rat.

X-radiation of the pituitary and the testes was the experimental technique. Pituitaries of ground squirrels were rayed at various times throughout the annual cycle with 7 to 4000 roentgen units. Pituitaries of various ages of rats were rayed with dosages up to 6000 roentgen units.

Testes of both species were x-rayed. In the ground squirrel this was done at appropriate times during the annual cycle.

Structures of the endocrine system and its end points were weighed and representative specimens were studied histologically.

Body weights were noted carefully at short intervals in the case of young rats whose pituitaries had been rayed.

As a result of these studies the following conclusions were reached:

1. The pituitary is extremely resistant to the effect of x-ray.

2. No good evidence of a "stimulating dose" of x-ray has been found in the work reported, nor in the reports of other laboratory workers.

3. The pituitary of the ground squirrel, *Citellus tridecemlineatus*, is especially unreactive when the animal is in the quiescent phase of its annual sex cycle.

4. The gonads of *Citellus* in the sexually inactive phase are likewise very resistant to x-ray.

5. The secretion of the gonadotropic hormone of the pituitary is inadequate to bring about recovery of gonads from x-ray injury within 40 days, during this inactive phase.

6. A differential susceptibility of the cell types of the anterior pituitary to x-radiation has been demonstrated. The alpha cells lose their characteristic granules, the cytoplasm shrinks, and the nucleoli increase in size enormously. The beta cells and chromophobes are apparently unaffected within the range of dosage used on the ground squirrel.

7. X-ray stimulates the sclerotization of the walls of the blood sinuses of the pituitary. An engorgement of these spaces with red blood cells accompanies this thickening of their walls.

8. These changes in the pituitary of the ground squirrel are without effect on the sex complex. A tentative explanation is suggested.

9. The growth rate of young rats is reduced by 50% following x-radiation of the pituitary with 1000 r. or more.

10. Two thousand r. or more applied to the pituitary of these young rats inhibits spermatogenesis.

11. Six thousand r. applied to the adult rat pituitary inhibits gametogenesis.

12. "Castration" or "signet-ring" cells are seen in these rayed adult rat pituitaries, beginning at dosage levels lower than those necessary to produce effects on the germ line.

13. In view of the profound effects obtained through large doses of x-ray and the apparent lack of effect of doses, such as are used medically, on the inactive pituitary, a note of caution is sounded concerning the indiscriminate use of the therapy in question, and doubt is thrown on its usefulness to "stimulate" an inactive gland.

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